Claim 6 stands rejected under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 1-10 and 13-16 of US 5,686,276 in view of Daniel et al (1992), in light of Daniel et al. (1995).

Claims 1-18 and 33 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatenetable over Claimsd 1-10 of US 5,821,092.

Claims 1-18 and 31-33 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-10 of US 5,633,362.

Claims 1-18 and 31-33 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-33 of US 6,013,494.

The remaining Claims are 2, 6, and 31.

Patentability Under 35 USC 112:

Claims 1-12, 14-18, and 31-33 stand rejected under 35 USC 112, first paragraph, as the specification, although found enabling for a process of generating 1,3-propanediol with a microorganism transformed with the *Klebsiella pneumoniase dhaB* gene, is regarded as not reasonably providing enablement for production of 1,3-propanediol by any microorganism transformed with any diol dehydratase gene from any other organism.

Claims 1, 3-5, 7-19, and 32-33 have been cancelled herein. Claims 20-30 were cancelled in the preliminary amendment. Claims 2, 6, and 31 are pending.

Claims 5,6,12, and 14 stand rejected under 35 USC 112, second paragraph, as being indefinite for not particularly pointing out and distinctly claiming the subject matter Applicants regard as the invention. Claim 6 remains pending in this application.

Claim 6 has been amended to clarify which exogenous genes are contained in the DNA fragment.

Patentability under 35 USC 102 (b):

Claims 1,3,-5-10, 18, and 33 stand rejected under 35 USC 102(b) as being anticipated by Daniel et al. The Examiner states that the the reference is anticipatory because it provides

transformed *E. coli* which expresses the *Citrobacter freundii dha* regulon and produces active glycerol dehydratase. Claims 2, 6, and 31 are pending in this application.

Daniel and Gottschalk (1992) disclose the anaerobic production of 1,3-propanediol from glycerol using an *E. coli* transformed with the *Citrobacter freundii* genes encoding the *dha* regulon. The genes (e.g., genes encoding glycerol dehydratase) from one enteric bacterium (*Citrobacter*) were transformed into and shown to function within a different enteric bacterium (*Escherichia*). Daniel et al. (*J. Bacteriol.* 177 (1995): 4392-401) teach the purification of 1,3-propanediol dehydrogenase and the production of 1,3-propanediol in wildtype *Citrobacter* where glycerol is the carbon source.

Since glycerol and/or dihydroxyacetone (DHA) are required for the expression of the *K. pneumoniae* enzymes leading to 1,3-propanediol production (glycerol dehydratase and 1,3-propandiol oxidoreductase), it is apparent that Daniel et al. relies on the natural (*Citrobacter*) regulatory system to control the DNA of the recombinant cosmid for the expression of glycerol dehydratase in *E. coli*.

It was unknown, until demonstrated by Applicants, that this gene can be made to function in microorganism species more distant from enteric bacterium. The inventors have demonstrated that these genes (e.g., genes encoding glycerol dehydratase) are fully functional in *Streptomyces*, *Bacillus*, *Pseudomonas* species and even eukaryotic species such as *Pichia*, *Saccharomyces*, and *Aspergillus*.

While the natural regulatory elements from one enteric bacterial (for example, the *K. pneumoniae* DNA within the cosmid pTC1) may function in another enteric bacteria (for example, *E. coli*), in 1995 the ordinary person skilled in the art would have appreciated the difficulty of expressing a multiple subunit, prokaryotic enzyme in eukaryotic organisms. The cited art clearly would not have suggested to a person skilled in the art the use of a recombinant eukaryotic microorganism for expression of a glycerol dehydratase enzyme.

Within the specification, the Applicants have specifically identified the multiple glycerol dehydratase genes: "Referring to SEQ ID NO:1, --- the open reading frame *dhaB1* encoding the alpha subunit glycerol dehydratase is found at bases 7044-8711; the open reading frame *dhaB2* encoding the beta subunit glycerol dehydratase is found at bases 8724-9308; the open reading frame *dhaB3* encoding the gamma subunit glycerol dehydratase is found at bases 9311-9736 ---".

Having first correctly identified the *alpha, beta,* and *gamma* subunits of glycerol dehydratase, the Applicants further coordinately expressed each of the subunits in yeasts (*Pichia*, Example 7; *Saccharomyces*, Example 9) and filamentous fungi (*Aspergillus*, Example 22) without the advantage of a natural, single bacterial promoter driving the expression of multiple polypeptides. This is not disclosed, taught, or suggested by Daniel et al.

Claim 2 specifically has glycerol as the carbon source and a number of specific microorganism species as the catalyst.

None of the host microorganisms contain an endogenous dehydratase. Also, none of these species are of the family *Enterobacteriaceae*. The point is that, at the time of the filing, heterologous dehydratase expression had only been demonstrated within Enterobacteriaceae (*Klebsiella* or *Citrobacter* genes into *Escherichia*). Furthermore, prior to this application, none of the specific microorganism species of Claim 2 had been shown to produce 1,3-propanediol from any carbon source.

Applicants have expressed glycerol dehydratase in
a) recombinant *Pichia* (eukaryote, yeast): Example 7;
b) recombinant *Saccharomyces* (eukaryote, yeast): Example 9;
c) recombinant *Streptomyces* (bacteria, gram positive): Example 15;
d) recombinant *Bacillus* (bacteria, gram positive): Example 17;
e) recombinant *Pseudomonas* (bacteria, gram negative, obligate aerobe): Example 20;
f) recombinant *Aspergillus* (eukaryote, filamentous fungi): Example 22;

Applicants have produced 1,3-propanediol from glycerol using
a.) recombinant *Pichia*: Example 7,
b.) recombinant *Saccharomyces*: Example 9;
c.) recombinant *Streptomyces*: Example 15, 16;
d.) recombinant *Bacillus*: Example 17,;
e.) recombinant *Pseudomonas*: Example 20;
f.) recombinant *Aspergillus*: Example 22.

Having responded to the Examiner's objections and rejections, Applicants respectfully request that the rejections be withdrawn, the claims reconsidered and the application passed to allowance.

Respectfully submitted,

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12 May 2003

Dated: 25 November 2002

Marked pages indicating the amendments, Brackets [ ] indicated deleted text; underlingin _____ indicates inserted text.

2. (Amended One Time) A bioconversion process to produce 1,3-propanediol comprising contacting, under suitable conditions, glycerol or dihydroxyacetone with a single recombinant microorganism having at least one exogenous gene [capable of] expressing a glycerol dehydratase enzyme, the microorganism selected from the group consisting of members of the genera *Aspergillus*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacter*, [*Salmonella*,] *Bacillus*, *Streptomyces*, and *Pseudomonas*.

6.(Amended One Time) The process of Claim [1] 2 wherein the microorganism is transformed with at least one exogenous DNA fragment encoding dhaB1, dhaB2, and dhaB3 and /or dhaT [whereby the organism expresses active dehydratase enzyme].

31. (Amended One Time) A recombinant eucaryote microorganism selected from the group consisting of yeast and filamentaous fungi, and expressing an exogenous glycerol dehydratase enzyme.